Nitrosative stress-based specific evaluation of creatine use in combination with aerobic running exercise at different speeds: a preclinical study in mice

A. TASKIN¹, H. CELIK², S. TASKIN²

¹Department of Nutrition and Dietetics, Faculty of Health Sciences, Harran University, Sanliurfa, Turkey
²Department of Physiology, Faculty of Medicine, Harran University, Sanliurfa, Turkey

Abstract. – OBJECTIVE: In acute and chronic aerobic exercise, skeletal muscle and liver are the main organs that adapt and regulate metabolic activity. The levels of nitrosative stress caused by exercise in these organs are extremely important in the continuity of exercise, its health-promoting benefits, and the evaluation of therapeutic efficacy. In this study, nitrosative stress levels were investigated in muscle and liver tissue of mice that were given low and high-speed aerobic running exercise and also received Cr supplementation.

MATERIALS AND METHODS: In this study, nitrosative stress levels were investigated in the muscle/liver tissue of 42 BALB/c mice that were given low and high-speed aerobic running exercise and creatine monohydrate (Cr) (40 mg/kg of diet daily) supplementation with exercise. The study included six groups with and without Cr supplementation, low-speed aerobic running, high-speed aerobic running, and no exercise. The mice in groups with low-speed and high-speed aerobic exercise with and without Cr supplementation were run on the treadmill for 8 weeks. Then, nitric oxide (NO·), nitric oxide synthase (NOS), and peroxynitrite (ONOO-) levels in muscle/liver tissue were measured by spectrophotometric method.

RESULTS: It was found that the nitrosative stress level in the groups that did low and high-speed aerobic running exercises increased compared to the group that did not exercise. It was found that NO· decreased NOS activity and ONOO- level increased in muscle tissues of low and high-speed aerobic exercise groups that received Cr supplementation compared to those that did not. However, NO· and ONOO- levels in liver tissue decreased while NOS activity did not change. The lowest level of nitrosative stress in both muscle and liver tissue was found in the low-speed exercise group receiving Cr supplementation.

CONCLUSIONS: Although supplements in exercise are an important component, the simultaneously measured nitrosative stress level is critical in determining the optimal exercise.

Key Words: Nitrosative stress, Exercise, Creatine, Supplementation.

Introduction

Exercise provides important evidence for the prevention of chronic diseases such as cardiovascular diseases, diabetes, cancer, hypertension, obesity, depression, and osteoporosis and the prevention of premature death. This makes it imperative to maintain a healthy lifestyle. The ability to sustain exercise, its health-promoting benefits, and its therapeutic efficacy are possible through the coordinated operation of oxygen-related metabolic pathways. This occurs through many multi-organ interactions, including heart, lungs, peripheral muscles, liver, and nervous system. The compatibility of these systems is attributed to the type, duration, intensity of the exercise, and the presence of ergogenic substances used.

High oxygen utilization and mitochondrial activities in the muscles during exercise can promote free radical production and create toxic effect. However, there is increasing evidence that the effects of free radicals are not only toxic, but also play an important role in cell signaling and the regulation of gene expression. The oxygen that is not completely metabolized or converted to radical form is the main source of more toxic reactive oxygen-nitrogen species. Throughout the oxidative
phosphorylation in mitochondria, electrons escaping from the complexes on the inner membrane react with O$_2$ to form the superoxide radical (O$_2^-$). Previous studies have suggested that O$_2^-$ generation is the main source of muscle free radical production and that more toxic radicals are produced from O$_2^-$ damaged muscle. There are studies reporting that radical production is formed in response to physiological stimuli and provides physiological adaptation. However, high-intensity exercise is known to produce excessive amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Nitric oxide (NO), one of the RNS, is a low-reactive molecule with an important physiological role in the regulation of vaso-motor activity and immunomodulatory pathways, as well as cellular mechanisms such as neuronal activity. However, in the presence of O$_2^-$, NO becomes toxic and forms peroxynitrite (ONOO$^-$), a stronger oxidant. ONOO$^-$ is formed directly without the need for enzymes. NO reacts with the O$_2^-$ faster than the endogenous superoxide dismutase enzyme, which converts the O$_2^-$ to hydrogen peroxide. Therefore, the kinetics of the reaction of O$_2^-$ with NO necessitates the formation of ONOO$^-$.

This will make the intensity of the exercise and the nitrosative stress level very important.

Timing of ergogenic supplementation may be an important strategy to prevent exercise-induced oxidative stress and improve physiological adaptation. Creatine supplementation is one of the most used ergogenic substances due to its properties in providing cellular energy and energy transfer by increasing the phosphocreatine pool. In addition to its effects on cellular energy metabolism, there is also evidence that it is effective in antioxidant and anti-inflammatory effects, managing dyslipidemia, improving glycemic control, and improving mitochondrial function. In our previous study, we found that Cr supplementation tolerates the generation of exercise-induced ROS, improves mitochondrial biogenesis, maintains oxidant-antioxidant homeostasis, and optimizes the transformation of this dynamic balance.

Studies have confirmed that prolonged and high-intensity exercise causes excessive oxidative stress in both blood and skeletal muscle, damaging biomolecules such as DNA, protein, and lipids. Therefore, regulation of oxidative and nitrosative cascades during exercise and determination of factors affecting oxidative/nitrosative activity will be very important. Although the effects of different-intensity exercise and creatine supplementation on oxidative stress have been investigated, their potential effects on nitrosative stress have not been investigated. In this study, nitrosative stress levels were investigated in musculus quadriceps femoris tissue and liver tissue of mice that were given low and high-speed aerobic running exercise and also received Cr supplementation.

**Materials and Methods**

**Chemicals**

Creatine monohydrate, N-(1-Naphthyl) ethylenediamine, sodium nitroprusside dihydrate, sulfanilamide, and phenol were purchased from Sigma (St. Louis, MO, USA). L-Arginine, sulfanilic acid, cadmium, glycine, NaOH, ZnSO$_4$, CuSO$_4$, and NaNO$_3$ was purchased from Merck (Darmstadt, Germany). Ketamine (Alfamine, Alfasan) and Xylazine (Rompun, Bayer) were purchased commercially.

**Animal**

This study protocol was approved by the Harran University Animal Experiments Local Ethics Committee (Approval No.: 2022/002/06) and conformed to the Guide for the Care and Use of Laboratory Animals. Animals were obtained from Harran University Animal Experimentation and Research Center, where all the experiments were carried out. 8-10 weeks old male, weighing 20 to 30 g BALB/c mice, were housed under standard conditions. In experimental applications, a minimum number of animals were used in accordance with the 3R principle (Replacement, Reduction, Refinement) aimed at protecting animals. In addition, only male animals were included in the experiment in order to eliminate the effectiveness of estrogen. 42 male mice were housed 7 mice/cage under 12 h light:12 h dark schedule in a temperature-controlled environment (20-22°C). The cages were made of transparent polyethylene material and were designed for viewing from the outside. The mice were housed in a well-ventilated room and allowed free access to diet along with water ad libitum. Mice in the exercise group that received creatine monohydrate (Cr) supplementation were fed at 40 mg creatine monohydrate per kg of diet daily, while the other groups were fed a standard rodent diet for 8 weeks.

To avoid the effects of circadian variation, the exercise experiments were carried out daily between 09:00 and 12:00. Mice fed with standard rodent diet and not exercising were included in the study as a control group. Each experimental group consisted of 7
mice. Mice were randomly divided into 6 groups; Group I: non-exercise control group (n=7). Group II: non-exercise control group plus Cr supplementation (n=7). Group III: the group in which low-speed aerobic running exercise was performed (n=7). Group IV: low-speed aerobic running exercise group plus Cr supplementation (n=7). Group V: high-speed aerobic running exercise group (n=7). Group VI: high-speed aerobic running exercise group plus Cr supplementation (n=7).

**Experimental Design**

The exercise protocol was applied for 8 weeks. It was applied every day at the same time, five days a week. The mice in Groups III, IV, V, and VI were subjected to running exercises on a customized treadmill with speed, duration, incline, and selectable acceleration settings. Before starting the exercise experiment, randomly selected exercise groups were given practice exercises at 4 m/min for 5 minutes daily for 1 week. Then, mice in Groups III and IV (Low speed aerobic running exercise groups) were run at 8 m/min for 30 minutes daily on a 0° incline until the end of the eighth week. The mice in Groups V and VI (high-speed aerobic running exercise groups) were run at a speed of 8-12-18-21 m/min at 0° incline until the 5th week. Running speeds were gradually increased. Then, the experiment was completed by running at a speed of 24 m/min at a 0° incline for 30 minutes until the end of the exercise protocol11,27. No exercise was applied to Groups I and II (sedentary lifestyle) (Figure 1).

**Tissue Samples Preparation**

The mice in all groups included in the experiment were sacrificed 48 h after the last exercise session. The mice’s skeletal muscle (m. quadriceps femoris) tissue and liver tissue were immediately dissected out and homogenized (10% w/v) in 0.1 M PBS, and centrifugation of homogenate was made at 10,000 g for 30 min. The supernatant was collected, and this fraction was used for nitrosative stress analysis. All procedures were performed at 4°C. The measurement of muscle and liver protein concentration was according to the µ drop plate method (Varioskan™ LUX; ThermoFisher Scientific). The results were expressed as mg/mL.

**Determination of Nitric Oxide (NO•) Level**

Nitric oxide levels were assayed by measuring total nitrite/nitrate concentrations (stable end products of NO•) in the m. quadriceps femoris muscle and liver homogenates of mice using diazotization or Griess assay method28,29. The method’s basic principle is based on the formation of azo compounds by the reaction of nitrite, an indirect indicator of NO, with α-naphthylamine and sulfanilic acid at an acidic pH. Later modifications resulted in higher sensitivity, reproducibility, and faster analysis times28. Griess reagent cannot detect nitrate formed by some reactions of NO•. For a complete analysis, nitrate in the reaction medium is reduced to nitrite with cadmium.

![Figure 1](image-url)
The NO· levels are calculated by comparing the absorbance of the azo chromogen solution to a calibration curve prepared with known sodium nitrite concentrations (1-100 µM/L). The results were expressed as nM/mgr protein. Experiments were performed in duplicate, and three independent experiments were completed.

**Determination of Nitric Oxide Synthase (NOS) Activity**

NOS activity was determined as previously described\(^2\)\(^9\)-\(^3\)\(^1\). First, 200 µl of 20 mM NO substrate L-arginine was added to 100 µl of tissue homogenate and incubated at 25°C for 1 hour. After incubation, for the diazotization reaction, 200 µl of 4 mM HCl, 20 mM sulfanilic acid, and 12.5 mM N-(1-naphthyl) ethylenediamine was added. After the second incubation of 10 minutes, the absorbance of the sample tube was measured spectrophotometrically at 540 nm against a blank tube to which no arginine was added. In this method, sodium nitroprusside was used as the chemical standard. The results are expressed as U/mgr protein. Experiments were performed in duplicate, and three independent experiments were completed.

**Determination of Peroxynitrite (ONOO·) Level**

The peroxynitrite assay modified by Al-Nimer et al\(^3\)\(^2\)-\(^3\)\(^4\) was determined as previously described\(^3\)\(^3\)-\(^3\)\(^4\). Briefly, to obtain a final volume of 2 ml, 10 µl of tissue homogenates were mixed with 5 mM phenol and 600 mM Angeli’s Salt in a 50 mM sodium phosphate buffer (pH 7.4). The mixture was incubated for 2 hours at 37 degrees in the dark, and then 15 µl of 0.1 M sodium hydroxide (NaOH) was added. After incubation, the optical densities of the samples at 412 nm were measured in a spectrophotometer. The yield of nitrophenol was calculated from \(ε=4,400\ M^{-1}cm^{-1}\). The results are expressed as nM/mgr protein. Experiments were performed in duplicate, and three independent experiments were completed.

**Statistical Analysis**

Analyses of data were performed using SPSS 25.0 package program (SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). All data were checked for normality with the Shapiro-Wilk test. All outcomes, including the measured concentrations of proteins, NO·, NOS, and ONOO· were evaluated statistically, and the final results were expressed as median-interquartile range. The multi-group comparison of nonparametric variables was conducted by the Kruskal-Wallis test, and pairwise comparisons were conducted using the Mann-Whitney U test. The data meet the statistical assumptions of the tests. The \(p\)-value <0.05 was considered statistically significant.

**Results**

The study included 42 mice, with seven in each group. During the nine-week experiment, there was no difference in weight gain between the groups that received and did not receive Cr supplementation.

**The Comparison of NO·, NOS, and ONOO· in Muscle Tissue Among the Experimental Groups**

Nitric oxide levels in Group IV and Group VI receiving Cr supplementation decreased according to Group III and Group V, which did not receive Cr supplements, but this was not statistically significant (Table I, Figure 2). In all groups, NO· levels increased gradually with Cr supplements and exercise severity, and this increase was found to be statistically significant (\(p<0.001\)). Cr supplementation increased NOS activity independently of exercise and exercise speed. NOS activity in Groups IV, V, and VI was significantly higher than in Groups I, II, and III (all \(p<0.05\)). There was no significant difference between the groups in terms of ONOO· levels in muscle tissue (\(p=0.448\)). Interestingly, ONOO· levels increased in exercise groups (Groups IV and VI) receiving Cr supplementation, but this increase was not significant (Table I).

**The Comparison of NO·, NOS, and ONOO· in Liver Tissue Among the Experimental Groups**

The NO· level decreased in the exercise groups (Groups IV and VI) that received Cr supplementation compared to the exercise groups (Groups III and V) that did not, and this was statistically significant (\(p=0.015\)). NO· level in group V was significantly higher than all other groups (all \(p<0.05\)) (Figure 2, Table II). There was no significant difference between the groups in terms of NOS activity in liver tissue (\(p=0.184\)). In the liver tissue as well as in the muscle tissue, the ONOO· level in the groups that received Cr supplementation was significantly lower than in the groups that did not receive Cr supplementation, and it was statistically significant (\(p=0.021\)). The ONOO· level in Group IV was significantly lower than in Groups I, III, and V. The highest ONOO· level was in Group V (Table II).
A new insight into the effect of exercise on nitrosative stress

Table I. Enzyme activity of NOS and levels of NO\textsuperscript{•} and ONOO\textsuperscript{–} of the groups in muscle tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO\textsuperscript{•} median (IQR) (nM/mgr Protein)</th>
<th>NOS median (IQR) (U/mgr Protein)</th>
<th>ONOO\textsuperscript{–} median (IQR) (nM/mgr Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.30 (0.13)</td>
<td>0.25 (0.21)</td>
<td>19.7 (1.4)</td>
</tr>
<tr>
<td>Group II</td>
<td>0.31 (0.16)</td>
<td>0.31 (0.16)</td>
<td>18.9 (4.2)</td>
</tr>
<tr>
<td>Group III</td>
<td>0.26 (0.14)</td>
<td>0.23 (0.25)</td>
<td>18.4 (1.6)</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.21 (0.09)</td>
<td>0.42 (0.25)\textsuperscript{a}\textsuperscript{g}</td>
<td>21.3 (1.9)</td>
</tr>
<tr>
<td>Group V</td>
<td>0.31 (0.09)</td>
<td>0.48 (0.35)\textsuperscript{a}\textsuperscript{b}\textsuperscript{h}</td>
<td>18.3 (5.1)</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.27 (0.07)</td>
<td>0.67 (0.48)\textsuperscript{a}\textsuperscript{i}</td>
<td>20.4 (6.7)</td>
</tr>
<tr>
<td><em>p-value</em></td>
<td>0.310</td>
<td>&lt;0.001</td>
<td>0.448</td>
</tr>
</tbody>
</table>

Nitric oxide (NO\textsuperscript{•}), nitric oxide synthase (NOS), peroxynitrite (ONOO\textsuperscript{–}). IQR: Interquartile range. Group I: Control; Group II: Control plus Creatin Monohydrate (Cr); Group III: Low-speed aerobic running exercise; Group IV: Low-speed aerobic running exercise plus Cr; Group V: High-speed aerobic running exercise group; Group VI: High-speed aerobic running exercise plus Cr. *: \textit{p}<0.05 was considered significant and obtained from Kruskal-Wallis; \textsuperscript{a}: \textit{p}<0.05, \textsuperscript{b}: \textit{p}<0.01, \textsuperscript{c}: \textit{p}<0.01 vs. Group I; \textsuperscript{d}: \textit{p}<0.05, \textsuperscript{e}: \textit{p}<0.01, \textsuperscript{f}: \textit{p}<0.01 vs. Group II; \textsuperscript{g}: \textit{p}<0.05, \textsuperscript{h}: \textit{p}<0.05, \textsuperscript{i}: \textit{p}<0.05 vs. Group III.

Figure 2. NO\textsuperscript{•} levels in muscle and liver tissue. A. Muscle tissue; (B) Liver tissue. Group I: Control; Group II: Control plus Creatin Monohydrate (Cr); Group III: Low-speed aerobic running exercise; Group IV: Low-speed aerobic running exercise plus Cr; Group V: High-speed aerobic running exercise; Group VI: High-speed aerobic running exercise plus Cr. *: \textit{p}<0.05 vs. all groups.

Table II. Enzyme activity of NOS and levels of NO\textsuperscript{•} and ONOO\textsuperscript{–} of the groups in liver tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO\textsuperscript{•} median (IQR) (nM/mgr Protein)</th>
<th>NOS median (IQR) (U/mgr Protein)</th>
<th>ONOO\textsuperscript{–} median (IQR) (nM/mgr Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.035 (0.01)</td>
<td>0.11 (0.06)</td>
<td>5.09 (1.3)</td>
</tr>
<tr>
<td>Group II</td>
<td>0.035 (0.01)</td>
<td>0.09 (0.08)</td>
<td>5.04 (1.1)</td>
</tr>
<tr>
<td>Group III</td>
<td>0.037 (0.01)</td>
<td>0.15 (0.09)</td>
<td>5.39 (1.1)</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.032 (0.01)</td>
<td>0.12 (0.11)</td>
<td>4.27 (0.4)\textsuperscript{a}</td>
</tr>
<tr>
<td>Group V</td>
<td>0.047 (0.01)\textsuperscript{a}\textsuperscript{e}\textsuperscript{f}</td>
<td>0.15 (0.08)</td>
<td>6.07 (2.5)\textsuperscript{a}\textsuperscript{i}</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.032 (0.02)\textsuperscript{g}</td>
<td>0.17 (0.12)</td>
<td>5.23 (1.3)</td>
</tr>
<tr>
<td><em>p-value</em></td>
<td>0.015</td>
<td>0.184</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Nitric oxide (NO\textsuperscript{•}), nitric oxide synthase (NOS), peroxynitrite (ONOO\textsuperscript{–}). IQR: Interquartile range. Group I: Control; Group II: Control plus Creatin Monohydrate (Cr); Group III: Low-speed aerobic running exercise; Group IV: Low-speed aerobic running exercise plus Cr; Group V: High-speed aerobic running exercise; Group VI: High-speed aerobic running exercise plus Cr. *: \textit{p}<0.05 was considered significant and obtained from Kruskal-Wallis; \textsuperscript{a}: \textit{p}<0.05, \textsuperscript{b}: \textit{p}<0.01 vs. Group I; \textsuperscript{c}: \textit{p}<0.01, \textsuperscript{d}: \textit{p}<0.05 vs. Group II; \textsuperscript{e}: \textit{p}<0.05 vs. Group III; \textsuperscript{f}: \textit{p}<0.01 vs. Group IV; \textsuperscript{g}: \textit{p}<0.05 vs. Group V.
Discussion

Nitrosative stress is caused by an increase in the rate of the reaction between nitric oxide and the superoxide anion radical, which results in the formation of peroxynitrite\(^{35}\). Peroxynitrite is a powerful oxidant and nitrating agent that causes protein, lipid, and nucleic acid damage. The radical power of peroxynitrite depends on the scarcity of endogenous antioxidants in the environment\(^{56}\). In addition, the superoxide radical formed during exercise is one of the most important sources of peroxynitrite and, therefore, nitrosative stress\(^{45}\). While the body itself is a source of ROS/RNS, the concentration of radicals increases depending on exercise intensity and causes greater damage. The body's antioxidant capacity is exceeded, and the proportion of reactive nitrogen species increases in relation to exercise intensity. Therefore, supplements to be taken with exercise are important. In this study, nitrosative stress levels were investigated in mice's muscle tissue and liver tissue that were given low and high-speed aerobic running exercises and received Cr supplementation.

Exercise is increasingly gaining acceptance in promoting health and preventing disease. It is also one of the leading factors that help increase human life expectancy\(^{37}\). Studies\(^{1,37,38}\) have shown that exercise can prevent a variety of life-threatening diseases, such as cardiovascular diseases, inflammatory diseases, obesity-related diseases, and certain types of cancer. Oxidative and nitrosative stress may be the direct or related factor that causes or prevents these diseases\(^{35,39}\).

Acute and strenuous exercise has been shown to increase nitrosative stress\(^{40}\). In the current study, NO and ONOO\(^{-}\) levels were found to increase with the speed of aerobic exercise in both muscle and liver tissue.

Exercise has been shown to alter redox homeostasis in nearly all organs, tissues, and bodily fluids\(^{41}\). Although the sources of radical production during exercise are still being debated, there is growing evidence that mitochondria are not the primary source. Intense and prolonged exercise, regardless of the radical sources, can cause oxidative damage to contracting myocytes, both proteins and lipids\(^{42}\). Non-muscle-damaging exercise and muscle-damaging exercise have been shown to have completely different effects on redox homeostasis. Non-muscle-damaging exercise causes changes in redox homeostasis several hours after exercise while muscle-damaging exercise causes changes that may occur and/or persist several days later\(^{41}\). The exercise model in our study and the nitrosative stress levels measured 48 hours after exercise show that redox homeostasis is impaired. Therefore, reducing and/or eliminating the damage caused by exercise in tissues and organs necessitates the use of supplements.

Cr is by far the most popular and scientifically studied dietary supplement. Cr supplementation has been shown to improve anabolic growth and exercise capacity, resulting in increased muscle mass and performance\(^{41}\). Our study found that Cr supplementation reduced the level of nitrosative stress in muscle tissue that occurs with exercise. It was found that nitric oxide levels increased with exercise intensity in the groups that did not receive Cr supplementation (Groups III and V) compared to those that received Cr supplementation. Especially low-speed aerobic exercise+Cr group was found to have the lowest nitric oxide levels. However, NOS levels, which catalyze nitric oxide formation, were higher and statistically significant in the groups that received Cr supplementation than in those that did not. Although there is an inverse relationship between NO and NOS levels in muscle tissue, it is thought that an important part of NO\(^{-}\) formed by NOS catalysis is ONOO\(^{-}\), a secondary metabolite of nitrosative stress. Therefore, in our study, the ONOO\(^{-}\) level increased in the groups that received Cr supplementation compared to those that did not. We think that the increase in ONOO\(^{-}\) level in the groups receiving Cr supplementation is due to the convenience of reaction kinetics and reaction direction and that Cr supplementation mediates this availability. The reaction of nitric oxide with superoxide to form peroxynitrite is about three times more effective than the superoxide dismutase reaction that catalyzes superoxide\(^{44}\). In cases where both types of radicals (NO\(^{-}\) and O\(_2^-\)) are present, ONOO\(^{-}\) formation is preferred. With this reaction, nitric oxide can reduce superoxide toxicity, while superoxide can reduce nitric oxide availability and inhibit physiological effects such as vasodilation. These results were also confirmed by studies on endothelial cells\(^{45,46}\). Our findings in skeletal muscle tissue support these results.

The liver is very important for the effects of exercise due to its metabolic effects on the whole organism and its contribution to the exercise muscles' energy supply. Recently, it has been shown\(^{47}\) that the liver plays an important role in redox status and inflammatory modulation during exercise. Understanding the effects of the exercise of different intensities on the liver and how this major metabolic organ adapts to these effects is important to expand our knowledge of the health benefits associated with exercise.
A new insight into the effect of exercise on nitrosative stress

There are limited studies \(^48,49\) investigating the effect of exercise on liver nitrosative stress levels. In these studies, it has been suggested that exercise causes an increase in NO· and NOS levels in liver tissue and causes significant changes in iron metabolism \(^48,49\). Indeed, exercise increases nitrosative stress in liver tissue. In this study, NO· and ONOO- levels in the liver tissues of the groups that did high and low-speed aerobic exercise (Groups III and V) were higher than those that did not exercise (Group I). On the other hand, NO· and ONOO- levels in the exercise groups that received Cr supplementation (Groups IV and VI) were lower and statistically significant compared to the exercise groups that did not receive Cr supplementation (Groups IV and VI). Cr supplementation with exercise reduced NO· and ONOO- levels in both low-speed aerobic exercise and high-speed aerobic exercise. In particular, low-speed aerobic exercise plus Cr supplementation was found to be important in reducing both NO· and ONOO- levels in the liver tissue in terms of reducing the nitrosative stress caused by exercise. It is thought that this effect of Cr may be due to its antioxidant properties \(^1\). In addition, it should be kept in mind that the liver may have reduced the level of nitrosative stress due to its feature as a detoxification organ and its role in nitrogen metabolism. However, conflicting data show that grape seed extract, which has antioxidant properties, increases NO· levels in the rat liver with exercise \(^5\).

Limitations

One of our limitations is that the superoxide level was not measured in this study. The superoxide radical formed during exercise is one of the important sources of peroxynitrite and, therefore, nitrosative stress. Measurement of superoxide radical level could be important to support our results.

Conclusions

While exercise affects the whole body, the main organs in regulating adaptation to exercise are the skeletal muscle and the liver. The effects of exercise on these organs are extremely important in terms of evaluating the benefits of exercise. We found that exercise increases nitrosative stress in muscle and liver tissue, regardless of the speed of aerobic exercise. The fact that Cr supplementation with exercise lower nitrosative stress in muscle and liver tissue is another significant conclusion of this study. In conclusion, while exercise triggers nitrosative stress in muscle and liver tissue, Cr supplementation has a protective role against nitrosative stress. This role of Cr supplementation was best observed with low-speed aerobic exercise. Finally, Cr supplementation should be provided with low-speed aerobic exercise to minimize possible nitrosative stress formations while benefiting from the therapeutic role of exercise in chronic diseases and its health-promoting effects.

Authors’ Contributions

Conceptualization, A.T., and H.Ç.; methodology, A.T.; validation, A.T., H.Ç., and S.T.; formal analysis, A.T.; resources, A.T.; data curation, A.T., H.Ç., and S.T.; writing—original draft preparation, A.T.; writing—review and editing, S.T.; supervision, H.Ç.; project administration, A.T., and H.Ç. All authors have read and agreed to the published version of the manuscript.

Informed Consent

Not applicable.

Funding

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Ethics Approval

The animal study protocol was approved by the Harran University Animal Experiments Local Ethics Committee (Approval No.: 2022/002/06).

Data Availability

The data presented in this study may be requested from the corresponding author on reasonable grounds.

Conflicts of Interest

The authors declare no conflict of interest.

ORCID ID

Abdullah Taskin: 0000-0001-8642-1567.
Hakim Celik: 0000-0002-7565-3394.
Seyhan Taskin: 0000-0002-3322-759X.

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